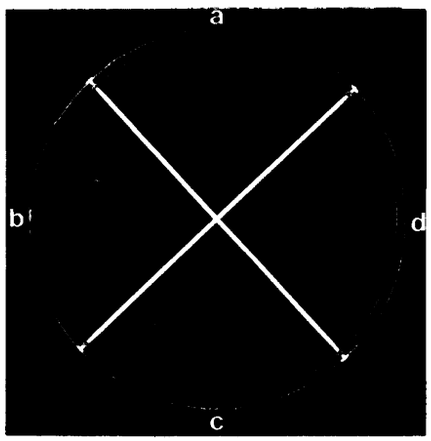


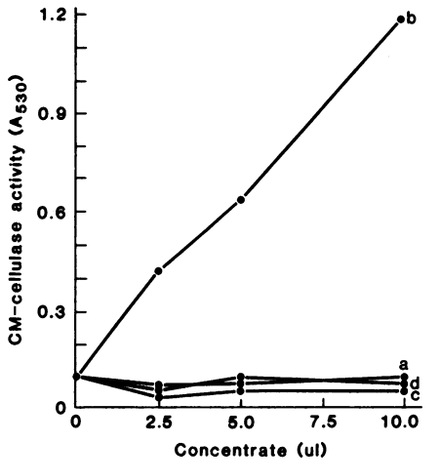


Fig. 3 (left). Secretion of CM-cellulase by yeast. Cells were grown at 30°C on selective medium (*26*), supplemented with 1.2 percent agar, 50 m*M* sodium phosphate, *p*H 6.8, and 0.9 percent carboxymethyl cellulose (Sigma, high viscosity). The colonies were washed off with water. The plate was rinsed with Congo red (2 mg/ml) for a few minutes, then rinsed with 1*M* NaCl. The unstained areas indicate hydrolysis of carboxymethyl cellulose to β(1 → 4)glucans that contain seven or fewer glucose residues (*27*). Cultures were strain 2OB12 containing either plasmid pOP (a), pL5.19 (b), pK2.4 (c), or pK1.3 (d). Transformants of strain SX34-4D were indistinguishable from those shown for strain 2OB12. Fig. 4 (right). Production of reducing sugar by yeast extracellular CM-cellulase. Yeast was grown at 30°C to a density of $1 \times 10^8$ to $2 \times 10^8$ cell/ml in selective medium (*26*), supplemented with L-tryptophan (20 μg/ml) for untransformed strain 2OB12. The supernatants were concentrated by ultrafiltration, dialyzed against water, and lyophilized. The material was dissolved in water, and samples were incubated for 1 hour at 37°C in 50 m*M* sodium phosphate, *p*H 6.8, containing 2 percent carboxymethyl cellulose (Sigma, low viscosity). One volume of 3,5-dinitrosalicylic acid (DNS) reagent (*10*) was added, the samples were boiled for 10 minutes, and the absorbance at 530 nm ($A_{530}$) was measured, with water used as a reference. The data were corrected for reactions stopped with DNS reagent at time zero. Cultures: a, untransformed 2OB12; b to d, 2OB12 containing plasmids pK2.4 (b), pK1.3 (c), or pL5.19 (d). Ten microliters of concentrate contained 28, 16, 18, and 26 μg of protein for cultures a to d, respectively. The CM-cellulase activity of culture b, based on reference to a glucose standard curve, is 1.6 units per milliliter of culture supernatant and 340 units per milligram of secreted protein, where 1 unit releases 1 μmol of reducing-sugar equivalents per minute at 37°C.

the β-polypeptide of preprotoxin results in a precursor conformation that interferes with normal processing.

These results demonstrate that *S. cerevisiae* can produce an extracellular CM-cellulase of bacterial origin and suggest that the effectiveness of this depends on the presence of an appropriate yeast leader peptide. *Cellulomonas fimi* grown on 0.1 percent Avicel produces 130 U of CM-cellulase per milliliter (*11*), a membrane-leaky mutant of *E. coli* C600 containing the cloned CM-cellulase gene produces 0.16 U/ml (*12*), and yeast containing plasmid pK2.4 produces 1.6 U/ml (Fig. 4). Since *C. fimi* produces a cellulase complex whose extracellular components act synergistically on cellulose (*13*), the level of CM-cellulase seen in yeast may compare more favorably to that of *C. fimi* than these numbers indicate. Two other examples of expression in *S. cerevisiae* of cloned endoglucanases from Gram-positive bacteria have been reported: an endo-1,3-1,4-β-D-glucanase from *Bacillus subtilis* (*14*), and an endo-1,4-β-D-glucanase or CM-cellulase from *Clostridium thermocellum* (*15*). In neither case was a yeast leader peptide incorporated into the cloned genes and in neither case was the endoglucanase secreted. Our results suggest that further constructions to incorporate genes for an extracellular exo-1,4-β-D-glucanase and 1,4-β-D-glucosidase will allow *S. cerevisiae* to utilize cellulose as a carbon source.


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## Neuroendocrine Response to Estrogen and Sexual Orientation

Gladue *et al.* (*1*) report that intravenous injections of 25 mg of Premarin (water-soluble, conjugated estrogens, especially sodium estrone sulfate) exerted a positive feedback action on serum luteinizing hormone (LH) within 48 hours in heterosexual women but not in heterosexual men. Premarin also stimulated a significant increase in serum LH in homosexual men 72 hours after estrogen treatment, and this LH concentration was between that observed in heterosexual males and that observed in heterosexual females. Gladue *et al.* thereby replicated earlier results of Dorner *et al.* (*2*), and concluded that the brain mechanism controlling the pituitary secretion of LH in homosexual men is somehow different from that in heterosexual men. We believe, however, that there may be an alternative explanation for these data.

Gladue *et al.* (*1*) refer to the fact that in females estrogen typically exerts a positive feedback effect on LH secretion during the ovarian cycle, whereas in males an LH response to exogenously administered estrogen is absent, which "presumably reflects 'male' brain differentiation" (*1*). This conclusion derives, however, from studies of nonprimate mammalian species (*3*). In macaques and

marmosets (*4*), estradiol stimulated similar increments in serum LH concentrations in both males and females, provided the animals were gonadectomized at the time estrogen was given. No such estrogen-induced surge in LH was seen in male macaques with gonads (*5*). Daily intramuscular injections of 1.5 mg of estradiol benzoate (EB) into four castrated men of unspecified sexual orientation caused a significant depression in serum LH, followed by a significant surge in LH secretion 120 hours after the onset of treatment (*6*). This EB-induced stimulation of LH secretion resembled the effects of estrogen in women with gonads (*7*). Daily EB injections caused smaller, but statistically significant, increases in serum LH within 96 hours in men with gonads ($n$ = 6, sexual orientation not specified) (*6*). These findings strongly suggest that in male primates (heterosexual men included) the neuroendocrine mechanism mediating positive feedback effects of estrogen on LH secretion is not appreciably "defeminized" during sexual differentiation.

The reported inability of estrogen to facilitate LH secretion in males with gonads in some primate species has been attributed to the potent inhibitory action of a testicular hormone on the responsiveness of the neuroendocrine axis to estrogen (*8*). The identity of this testicular factor is not known, although evidence from male macaques (*8*) suggests that it is neither testosterone nor dihydrotestosterone. It is possible that the heightened concentrations of LH observed by Gladue *et al.* (*1*) in homosexual males 72 hours and 96 hours after treatment with Premarin reflect a selective reduction in the testicular secretion of this inhibitory hormone in response to a direct testicular action of Premarin. This possibility must be ruled out before it can be concluded that the brain or pituitary mechanisms regulating LH secretion in homosexual men respond differently to estrogen than those in heterosexual men.

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Psychoendocrine studies in humans are rarely singularly definitive and all-conclusive, particularly in such a complex research area as behavioral psychobiology involving the hypothalamic-pituitary-gonadal axis. In our report (*1*) the main objective was to experimentally evaluate earlier suggestions by Dorner and his colleagues that a neuroendocrine response difference might exist related to differences in sexual orientation. It was those earlier reports that suggested adult LH response differences might reflect subtle differences in prenatal sexual differentiation and that those developmental influences were on the central nervous system. We found a differential response pattern in LH among the homosexual men that suggests a neuroendocrine responsiveness between that of the heterosexual men and the heterosexual women. We also discovered that some testicular differences might exist because testosterone concentrations also differed when estrogen was administered. We noted that those findings invite "the idea that there may be physiological developmental components in the sexual orientation of some homosexual men" and that "even though a developmental relation between neuroendocrine response and sexual orientation is not certain, our findings are not inconsistent with such an interpretation" (*1*). Our objective then, and now, is to understand those physiological components that may be involved in psychosexual development.

However, there can be alternative explanations and interpretations of these data, as we cautioned in our report. The explanation offered by Baum *et al.* is also appropriate. We are aware of the possibility that we may have measured indirectly a difference not at the hypothalamic but at the gonadal level, in which case a difference in some (as yet unidentified) testicular factor might account for a selective enhancement of pituitary LH.

Differences in LH and testosterone responsiveness in gonadally intact men of different sexual orientation are findings that invite further evaluation. That testicular functioning or factors might be responsible for such neuroendocrine response differences related to sexual orientation would still leave unanswered the question of the origin of such differences, be it testicular or hypothalamic.

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